

## CLAIMS

What is claimed is:

1. A method for automatic inspection of a microarray slide, comprising the steps of:

obtaining a gray scale image of the microarray slide, wherein the microarray slide comprises one or more blocks containing arrays of spots;

binarizing the gray scale image by selecting a threshold;

morphologically dilating the binarized image so as to generate a merged component;

calculating rotational offset and translational offset of the merged component so as to align the gray scale image with a reference image; and

determining qualities of the spots on the microarray slide by using threshold methods.

2. The method of claim 1, wherein the gray scale image is captured by a detection means.

3. The method of claim 2, wherein the detection means is selected from the group consisting of a charge-coupled device (CCD), a charge injection device, a photodiode array and a scanner.

4. The method of claim 3, wherein the detection means is a CCD camera.

5. The method of any of claims 1-4, wherein, in the binarization process, the threshold is predetermined by computing the histogram of the gray scale image, thereby the spots are separated from background for having intensity values exceeding the threshold.
6. The method of any of claims 1-5, wherein, in the morphological dilation process, the dilation operation uses any odd number matrix that is computational feasible.
7. The method of claim 6, wherein the matrix is a 3 x 3 one where the number of dilation operation is 8.
8. The method of claim 6, wherein the matrix is a 5 x 5 one.
9. The method of claim 6, wherein the matrix is a 7 x 7 one.
10. The method of any of claims 1-9, wherein the rotational offset is calculated by using any algorithms that can calculate the rotational offset of an object having a rectangular shape with a boundary.
11. The method of claim 10, wherein the rotational offset is calculated by using Sobel operation, where the magnitude of the edge of the image in the vertical direction and horizontal directions will be denoted as Gy and Gx, where Gy is determined by using Sobel Y mask of:

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-1	-2	-1
0	0	0
1	2	1

The G<sub>x</sub> is determined by using Sobel X mask of:

-1	0	1
-2	0	2
-1	0	1

The above edge magnitude for each image pixel is computed by computing G for each edge point by:

$$G = |G_x| + |G_y|$$

The orientation will be determined as:

$$\tan^{-1} (G_y / G_x)$$

The rotational offset is computed by getting the maximum number of edge points that give the same edge directions.

12. The method of claim 10, wherein the rotational offset is calculated by using second order moment analysis, where the central moment needs to be computed, as given in the formula below;

$$\mu_{ij} = \sum \sum (x - \text{Cen}_x)^i (y - \text{Cen}_y)^j P(x, y)$$

thereby the rotational offset can then be computed based on the central moments, as follows:

$$\theta = 0.5 \tan^{-1} [2\mu_{11} / (\mu_{20} - \mu_{02})]$$

13. The method of any of claims 1-12, wherein the translational offset can be calculated by any algorithms that are suitable for calculating the vertical and horizontal distances between the coordinate of one block and another reference block.

14. The method of claim 13, wherein the translational offset is calculated by using first order moment analysis, where the general moment formulation is given in the formula below:

$$M_{i,j} = \sum \sum x^i y^j P(x,y)$$

where  $i, j$  are the moment indices;  $x$  is the  $x$ -coordinate of the pixel,  $y$  is the pixel's  $y$ -coordinate, and  $P(x,y)$  is the pixel's intensity; thereby the centroid  $X$ , denoted by  $Cen\_X$  can be computed as:

$$Cen\_X = M_{1,0} / M_{0,0}$$

and, the centroid  $Y$ , denoted as  $Cen\_Y$  can be computed as:

$$Cen\_Y = M_{0,1} / M_{0,0}$$

therefore, the determination of centroid  $X$  and centroid  $Y$  will give the translational offset.

15. The method of any of claims 1-14, wherein the qualities include spot size, spot types and overlapping strengths.

16. The method of claim 15, wherein the spot size is determined by selecting a region of interest from the gray scale image; binarizing the

selected region on the basis of a threshold selected by a user; and determining the spot size on basis of pixel count.

17. The method of any of claims 1-16, wherein the types of spots include normal spots, weak spots, missing spots, and overlapping spots.

18. The method of claim 17, wherein the determination of whether a spot is normal, weak or missing comprises the steps of selecting a region of interest from the gray scale image; establishing multi-level thresholds; and determining the type of a spot on the basis of its position in relation to the multi-level thresholds.

19. The method of claim 18, wherein the thresholds are determined by methods selected from the group consisting of local threshold method, global threshold method and universal threshold method.

20. The method of claim 17, wherein the linkage strength of the overlapping spots is determined by image intensity and the connectivity between spots, and any linkage between two spots is designated as one of the eight directional linkages: N, S, E, W, NE, NW, SE and SW.

21. The method of any of claims 1-20, wherein the microarray slide is a cDNA microarray slide.

22. A system for automatic inspection of a microarray slide, comprising:

a keyboard for instructions/data input;

a display for showing images;

an image capture unit for capturing a gray scale image of the microarray slide;

a slide holding and transporting means for delivering the microarray slide to the image capture unit for image capturing;

a control means for controlling the operation of the slide holding and transporting means; and

a processing unit for receiving instructions/ data from the keyboard, sending image signals to the display, receiving the image data from the image capture unit, and sending controlling instructions to the control means;

wherein the processing unit is electrically connected to the keyboard, the display, the image capture unit and the control means via separate bus lines.

23. The system of claim 22, wherein the image capture unit comprises a detection means for capturing the image of the slide, and a light source for providing the slide with illumination.

24. The system of claim 23, wherein the detection means is selected from the group consisting of a charge-coupled device (CCD), a charge injection device, a photodiode array, and a scanner.

25. The system of claim 24, wherein the detection means is a CCD camera.

26. The system of claim 23, wherein the light source is a directional frontal lighting system.

27. The system of any of claims 22-26, wherein the processing unit comprises a system memory and application programs, wherein the application programs are capable of conducting the inspection methods of claims 1-21.

28. The system of claim 27, wherein the application programs include excel i/o database.